de Jong et al., http://www.jcb.org/cgi/content/full/jcb.201112135/DC1

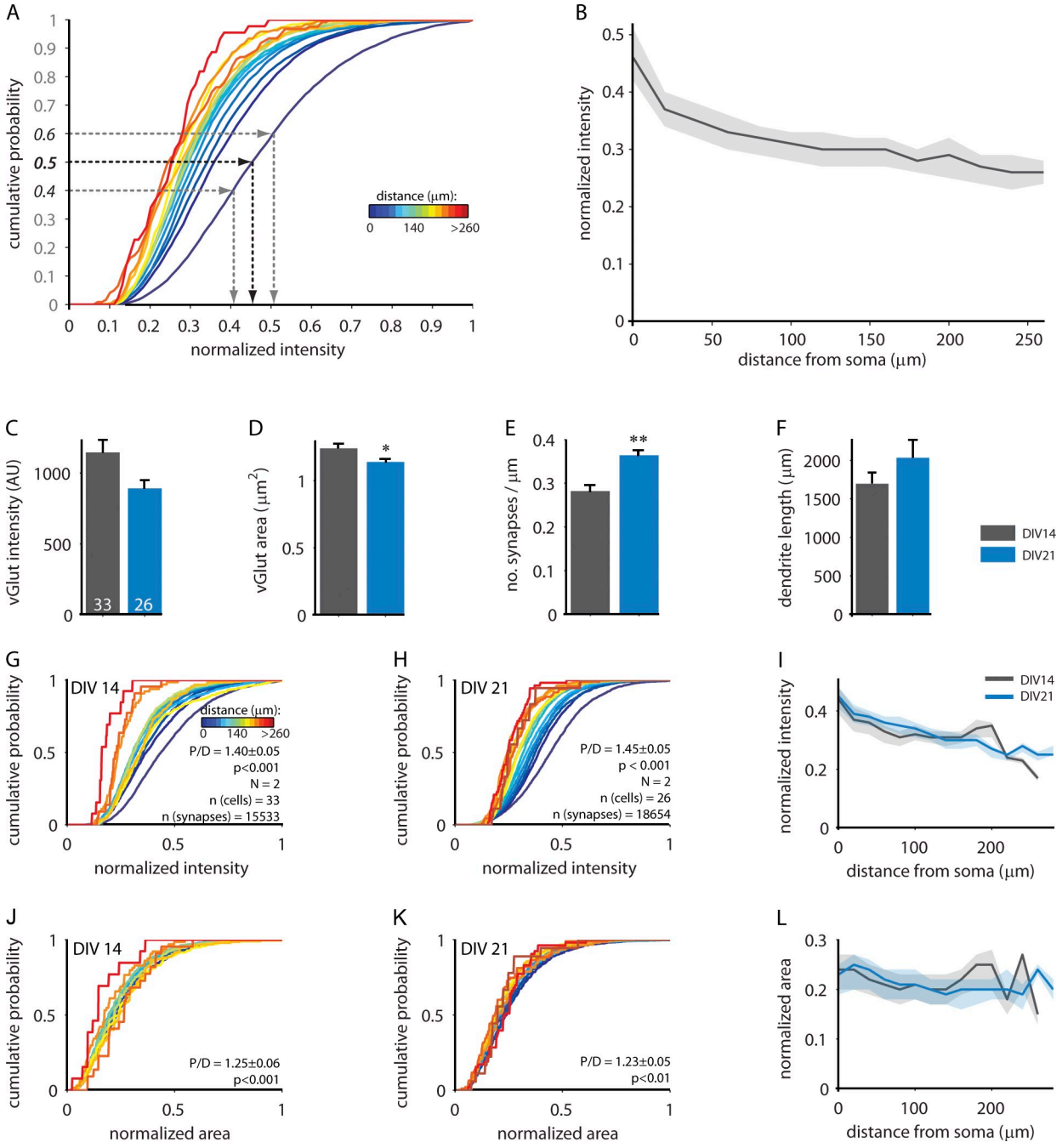

Figure S1. **Distance-dependent scaling of total pool size is independent of neuronal development.** (A) To compare distance-binned histograms of different experimental groups, we made profiles of each histogram. For each distance bin, we took the fluorescence intensity that corresponds with a cumulative probability of 0.4, 0.5 (the median), and 0.6, which is shown in B. (B) Profile of the distance-binned histogram in A. (B, I, and L) Solid line is the median, and shaded area represents the 0.4–0.6 boundaries. (C) Mean synaptic vGlut intensity per cell for neurons fixed at DIV 14 or DIV 21. Numbers refer to the number of observations. (D) Mean synapse area. (E) Mean synapse density per cell. (F) Mean dendrite length per cell. (G) Cumulative histograms of vGlut intensity of cells fixed at DIV 14, grouped by distance from the postsynaptic soma. Intensity was normalized to the largest synapse formed on the cell. The legend applies to H, J, and K. (H) As in G, for neurons fixed at DIV 21. (I) Comparison of histograms in G and H. (J) Cumulative histograms of area of synapses in E. (K) Cumulative histograms of area of synapses from H. (L) Comparison of histograms from J and K. Error bars represent means ± SEM. *, $P < 0.05$; **, $P < 0.01$. AU, arbitrary unit; N, number of experiments.

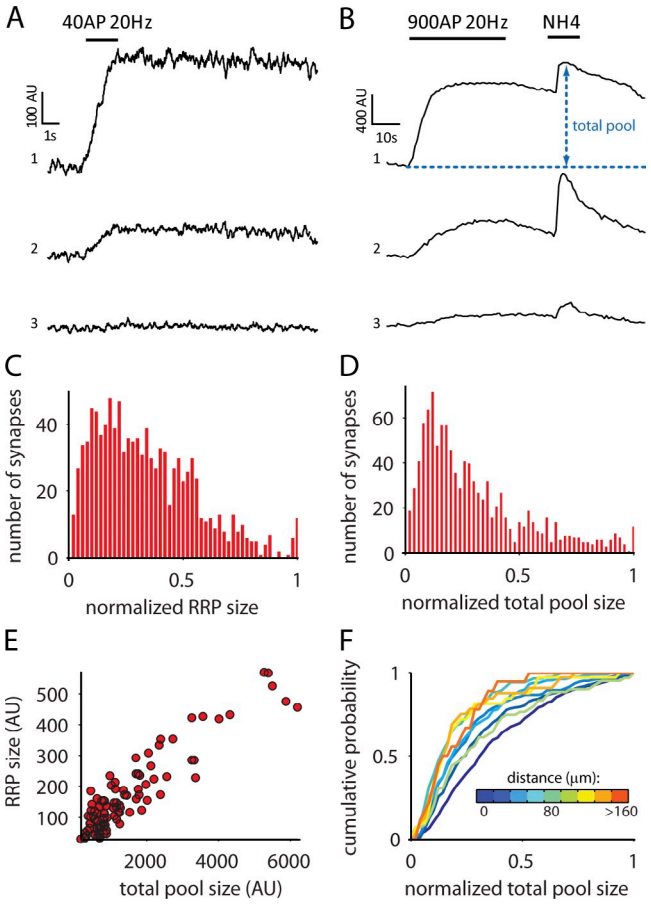

Figure S2. **Total vesicle pool size is related to RRP size and depends on synapse position.** (A) Representative traces of SypHy fluorescence from single synapses stimulated with 40 APs at 20 Hz to release the RRP. (B) Traces from the same synapses as in A, stimulated with 900 APs at 20 Hz to release the releasable pool and NH_4^+ to visualize the pool of unreleased vesicles. (C) Histogram of the RRP size as measured from the SypHy fluorescence. (D) Total pool size from the same synapses in C, measured from the response to 900 APs + NH_4^+. (E) Typical example of the relationship between RRP size and total pool size for synapses formed by one neuron. (F) Relationship between distance from the postsynaptic soma and the size of the total vesicle pool. Data are from 12 cells (1,066 synapses); $n = 3$. AU, arbitrary unit.

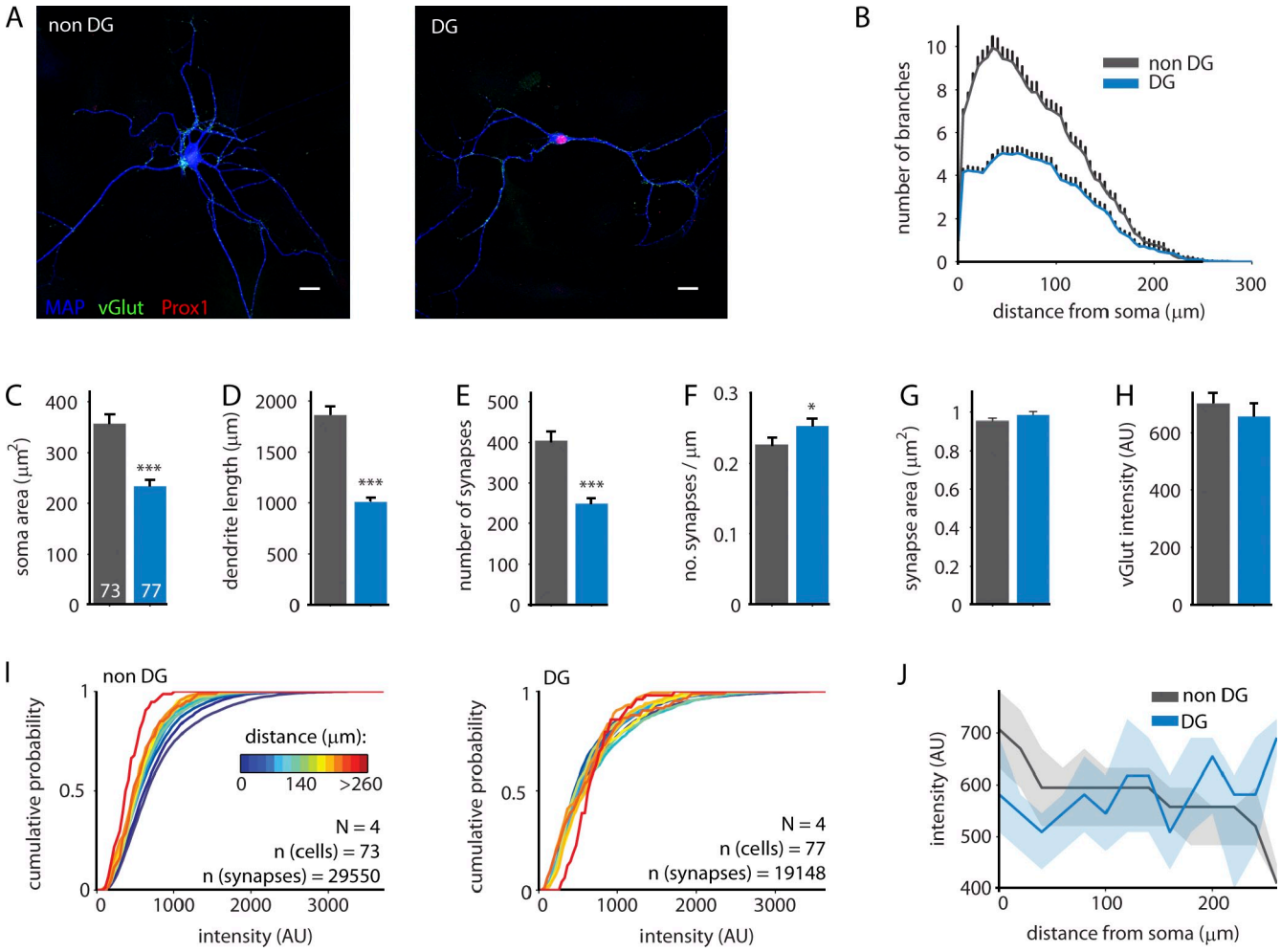

Figure S3. **DG granule cells retain their identity in culture and show strongly reduced distance-dependent scaling.** (A) Example images of a dentate gyrus (DG) cell and non-DG cell in culture. Bars, 20 µm. (B–H) Morphological characterization of DG and non-DG cells. (B) Sholl analysis of dendritic branching. The legend applies to C–H. (C) Surface area of the soma. (D) Total dendrite length in the field of view. (E) Number of synapses in the field of view. (F) Synapse density (number of synapses per micrometer of dendrite). (G) Surface area of synapses. (H) Mean vGlut intensity of all synapses. Data were obtained from 73 non-DG and 77 DG cells; $n = 4$. All data are expressed as means. Error bars represent SEM. (I) Cumulative histograms of absolute vGlut intensity of DG (right) and non-DG cells (left). Synapses were grouped by distance from the postsynaptic soma in bins of 20 µm. (J) Comparison of the histograms in I. Solid line is the median, and shaded area represents the 0.4–0.6 boundaries. *, $P < 0.05$; ***, $P < 0.001$. AU, arbitrary unit; N, number of experiments.